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INTRINSIC ORGANIZATION OF CEREBRAL TISSUE IN  
ALERTING, ORIENTING AND DISCRIMINATIVE RESPONSES

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Running Title: Intrinsic Organization of Cerebral Tissue

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# INTRINSIC ORGANIZATION OF CEREBRAL TISSUE

W. R. Adey

If our studies of brain functions are to lead ultimately to an understanding of the mechanisms of storage of information in cerebral tissue, and equally importantly, to ways in which its retrieval occurs, we must unrelentingly pursue processes which might have a causal relation to, and thus uniquely characterize, the laying down of a memory trace. It has not been clear to what extent, if any, electrical events in nervous tissue might bear a causal relationship to the initiation of those structural modifications in and around cerebral neurons that are presumably the basis of information storage.

In these circumstances, one might seek evidence in certain of the electrical signatures derived from brain tissue, that would reveal their preferential relation to phenomena of storage of information, as opposed to its mere transaction. That we should seek such differences should be made clear at the outset, for it will remain the central theme of this review. It may well be a herculean task, challenging the limits of current technologies and inviting our most earnest pursuit of new transducing methods in brain tissue. Yet without such a philosophy, there is no reason to suppose that even the most earnest study of ever finer details in synaptology and axonology will afford us a frame for observation and comprehension of this most unique quality of brain tissue. The functional correlates of information storage demand consideration of an anatomical frame that might spell out with equivalent clarity structural interrelations in cerebral tissue consistent with findings in physiological studies of learning mechanisms.

Whether we have succeeded at this time in establishing even a minimal basis for the structural and functional basis of information storage in brain tissue must remain for future investigation. We shall review even finer evidence relating the wave processes in cerebral tissue both to intracellular events, and to specifiable behavioral responses in alerting, orienting and discrimination;

and at the cellular level, the relations between intracellular waves and those recorded grossly from a domain of tissue in the EEG. In the latter area, we shall examine the premise that mere possession of a 'wiring diagram' of characteristically simpler structures, such as the cerebellum or hippocampus, might provide us with that flash of insight by which the very fundamentals of cerebral processes might stand revealed. Hopefully, we shall turn away from such notions, recognizing in both structural and functional organization of cerebral tissue aspects of uniqueness that would effectively delineate it from other quite complex central neural structures, not so endowed with a capacity for ready storage and retrieval of information.

In the very recognition of such a possibility, has come an awareness that modification of state consequent upon information storage may not be the exclusive prerogative of the neuronal compartment in cerebral tissue. Impedance measurements in cerebral tissue have a regional specificity in changes that accompany both alterations in broad general states of sleep and wakefulness, as well as in brief impedance transients occurring in alerting, orienting and discriminative responses. Further qualitative identification of the origins of these impedance changes has been gleaned following degenerative removal of the bulk of neuronal elements from cerebral nuclei, and has directed our attention to their possible genesis in mucoproteins and mucopolysaccharides distributed in intercellular fluid, where divalent cations, such as calcium, may modulate these macromolecular configurations, and thus indirectly influence ionic fluxes across the neuronal membrane.

1. Salient aspects of a tricompartamental model of cerebral tissue.

- a. The neuronal compartment

Nerve cells in mammalian cerebral cortex are arranged in a pattern characteristically described as laminar. The degree of orderliness in this layering varies from one cortical region to another, and is often a highly

subjective criterion (61). A unique and characteristic feature of cerebral cortex, on the other hand, and also of cerebral ganglia of many invertebrates, is the great overlap of the dendritic branches of one neuron with those of adjacent neurons, with dendro-dendritic contacts as close as in synaptic junctions (59). Possible functional interactions which might be initiated in this way are now being investigated, although numbers and locations of such contacts on any particular neuron remain unknown. Nevertheless, it is necessary that we consider such structural relations, since they may determine the degree of certain aspects of "coupling" between a neuron and others in its vicinity, in their mutual interaction in certain slow wave processes to be discussed below.

b. The neuroglial compartment.

The interrelations between neuronal and neuroglial elements in cerebral tissue has been the subject of elegant reviews elsewhere in this symposium. Only certain features will be emphasized here. It appears indubitable that by their intervention between nerve cells and the vascular apparatus, neuroglial cells can exercise a regulatory function on neuronal metabolism. The neuroglial envelope has been described by some as essentially complete around individual neurons, while others have noted a more restricted "packetting" of glial elements around synaptic terminals, and with relatively bare intervening areas(52). Enzymatic localization has been described in adjacent membranes where neuronal and neuroglial cells are in contact, and similarly, where neuroglial elements are in contact, but not where neuronal elements adjoin one another (18).

c. The extracellular space.

There remains for consideration a third compartment, the extracellular, traditionally given scant attention in most histological descriptions, but which may rank equally with the other two in functional significance. This

compartment might be considered of little importance if our notions of cerebral organization required it merely to contribute sodium ions. The size of the extracellular compartment has been variously estimated, but recent cytologic and chemical evidence has suggested a larger space than earlier electron microscope studies had indicated, and probably occupying as much as 15 to 20 percent of the cerebral volume (40). In sections of material prepared in ethylene and propylene glycol, and in the absence of calcium salts, Pease (54) has disclosed substantial amounts of material in intercellular clefts in cerebral cortex, not revealed in classical electron micrographs prepared from fixed material. This substance stains strongly with phosphotungstic acid at pH 3.5, thus differing from typical mucopolysaccharides, and its chemical identity remains undisclosed.

Movements of ions in the extracellular space will be substantially modified by the presence therein of macromolecules exhibiting ion-binding and fixed charge characteristics (46,70) so that it is not possible to model ionic behavior in the perineuronal environment from consideration of a mere aqueous solution permitting unimpeded flow. Impedance phenomena to be presented below require consideration of such macromolecules in terms of dynamic interactions with cellular elements that they surround. Conductance characteristics in the narrow clefts between neuronal membranes and adjacent neuronal and neuroglial elements will depend on their macromolecular content.

We may consider the extracellular compartment and its macromolecular systems as susceptible to ionic fluxes from neuronal elements on the one hand and neuroglial on the other. Moreover, by the very nature of these macromolecular systems in this middle zone, which are exceedingly susceptible in their molecular conformation and ion-binding characteristics to the presence of divalent cations, such as calcium, they may exert an important influence on ionic exchanges across

neuronal and neuroglial membranes (10). Bennett (19) has termed these macromolecular envelopes "glycocalyces," and has suggested for them a direct role in determining differential entry of sodium and potassium to positions close to the plasma membrane. Others have proposed a role restricted to pinocytotic transfer of vesicular material, and specifically excluding a role in ionic mechanisms (20).

It is proposed to consider this intercellular material from two aspects; firstly, as a source of fixed charges on the membrane surface, responsible for electrokinetic phenomena in the presence of an external EMF, and secondly, as the probable site of impedance changes in cerebral tissue accompanying transient alterations in its physiological state, including the recall of stored information.

d. The presence of fixed charge in the cell membrane.

The ability of macromolecules at the cell surface to exhibit fixed charges has been extensively tested in our laboratory by Elul (30,31), in movement of cells exposed to focal electric fields generated in the vicinity of micropipettes. Neurons, glia, and connective cells in tissue culture, as well as malignant cells and human erythrocytes in suspension, move towards the pipette when current is passed out of it, and repelled by passage of current in the opposite direction. (Fig.1)

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The focal electric field was generated by passing  $5 \times 10^{-7}$  to  $1 \times 10^{-5}$  A out of the micropipette, and induced movement of cells at a distance of 5 to 50  $\mu$  from the electrode tip. With cells embedded in culture, only local deformation of the membrane was observed, but once set free from the culture, cell movement replaced local deformation. Metabolic poisons had no effect on the movement, nor was it disturbed by rupture of the cellular membrane. Isolated membrane fragments showed movements similar to intact cells.

Such observations require careful exclusion of certain artifacts if the movements are to be attributed to a net membrane charge. Elul points out that the movements are not dependent on concentration of solutions in the micro-pipettes nor on the composition of the culture medium. The possibility of artifacts due to the proximity of the focal electrode were considered and excluded, including electroosmosis, and electrophoretic effects due to chemical potential gradients, or electric potential gradients or to electro-chemical gradients. It is therefore postulated that the movement is caused by a force acting on cells placed in the electric field. Such a force might arise in two ways; either by a difference between the dielectric constants of a neutral solid body and that of the medium in which it is placed, or by the presence of a net electric charge on the solid body. Analysis of the former possibility indicates that cells would exhibit a tendency to move to the locus of minimal electric field intensity, or in other words, to drift away from the focal electrode regardless of current polarity.

In summary, the only feasible mechanism appears to be that involving net electric charge, and subject to Coulomb's law. These charges must be firmly bound to the structural matrix of the membrane, since they persist in incubated membrane fragments. In such a scheme, selective permeability in the cell membrane may be envisaged as a two-step process. There would be an accumulation at the external surface of the membrane of potassium ions above their bulk concentration, and at a higher ratio to sodium ion concentration than in the solution. Transfer of potassium ions across the membrane would ensue by one of the mechanisms widely proposed for membrane transport, be it either by the Donnan mechanism, or a "pump," or an ionic exchange mechanism. It may be emphasized that in such a two-step mechanism, the requirements in ionic specificity would be a function of the macromolecular groupings, rather than of the intrinsic metabolic functions of the membrane.



## 2. The genesis of electric waves in brain tissue and their relation to cell firing.

In extracellular records, paired microelectrodes separated by only 30  $\mu$  record a spontaneous electroencephalogram of apparently normal form and amplitude (28). Moreover, no similarity could be found between monopolar and differential derivations of both spikes and slow waves recorded simultaneously with such small tip separations. This emphasizes that the dimensions of cortical dipoles generating the EEG are not necessarily larger than the dimensions of single cells.

### a. The generation of neural waves.

In further investigations by intracellular recording, Elul (29) noted that, although no EEG could be recorded with a liquid-filled micropipette on approaching the membrane of a single neuron with recording sensitivities in the low millivolt range, large, rhythmic waves were seen immediately after penetration in unanesthetized cortical neurons, superimposed on the resting membrane potential (Fig.2). These waves have an amplitude of 5 to 15 millivolts, and are hundreds of times larger than the EEG recorded in adjacent extracellular tissue or on the cortical surface. Similar findings in neocortical neurons have also been reported by Creutzfeldt, Fuster, Lux and Nacimienta (24) and Jasper and Stefanis (43), and in hippocampal neurons by Fujita and Sato (34).

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Autospectral analyses of intracellular waves and the EEG recorded grossly from the same region have indicated a strong general relationship between the two phenomena, both in sleep states with large regular slow waves and in the rapid wave trains of the aroused state. The high resistance of the neuronal membrane in relation to the low resistivity of the enclosing extraneuronal medium substantially reduces the magnitude of the EMF appearing in the extraneuronal medium through transmembrane current flow from intracellular generators.

Available data support the view that this attenuation would be at least 100:1, and are compatible with the observed EEG amplitudes of 50 to 200  $\mu\text{V}$  at the cortical surface (Fig. 3).

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Despite the similarities in autospectral density contours between intracellular waves and the gross EEG, calculations of cross-spectral functions, and particularly the coherence, between the two trains clearly reveal that the gross EEG does not arise from the simple summation of phase-locked contributions from the intracellular wave process. Levels of coherence remain extremely low over long periods. (Fig. 4) Elul's studies have indicated that the contributions from the intracellular generators occur on a statistical basis. The cortical generators appear to meet the requirements of the central limit theorem of Cramer (23), exhibiting individual amplitude distributions, which are not linearly related, possess a mean, and a finite standard deviation (48). Elul concludes that the EEG may be accounted for as the normal distribution ensuing from a combination of activity of non-linearly related neuronal generators, with frequency characteristics of individual generators relating strongly to the gross EEG, but with loss of phase relations in the process of summation.

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If such gross rhythmic waves arise in summed activity of neuronal generators, it is also necessary that we consider ways in which individual generators may be coupled in constantly varying degrees to other generators in the system (6,32). We have therefore investigated amplitude histograms of the gross EEG as an indicator of the cooperative behavior of neuron populations. Whereas amplitude histograms of the gross EEG tended to reach a normal distribution, intracellular wave activity was not distributed in a Gaussian fashion. If then, the gross EEG is derived through spatial summation of individual generators having non-Gaussian amplitude distributions, this is feasible, according to the central limit theorem, only if linear correlation is limited between the individual generators.

Recordings in human subjects with surface electrodes and electrodes chronically implanted in the hippocampus and amygdala, have shown that, although convergence to Gaussian does occur in healthy brain tissue, it is not perfect and markedly decreases during performance of demanding mental tasks. Since the sampled cortical neurons showed non-Gaussian activity even when the gross EEG was extremely close to Gaussian, it must be concluded that the changes observed in distribution of the gross EEG under varied behavioral stimuli cannot be wholly attributed to changes in amplitude distribution of the unitary generators. Rather, these changes in the amplitude distributions of the gross EEG could arise in terms of increased or decreased correlation between individual neurons in the sampled population. If this interpretation is correct, amplitude histograms of the EEG would provide an estimate of cooperative behavior of neuron populations.

b. The relationship of neuronal firing to waves and evoked potentials.

The firing of the neuron in relation to evoked potentials or induced wave trains in the same domain of tissue appears complex, (33), as is the simultaneously recorded activity of neurons in a single region after a stimulus (15,63). Fox and O'Brien (35) have found that variations in firing rate of a single cortical unit follow a contour similar to the evoked potential in the same region, but only after as many as several thousand repetitions of the stimulus. These findings may be interpreted in support of the view that the firing pattern of the individual neuron bears only a statistical relationship to the behavior of the integrated neuronal populations, as manifested by the essentially constant contour of the evoked potential.

In intracellular records, the firing of the neuron occurs near, but not necessarily at, the peaks of the depolarizing phase of the intracellular wave (5). However, we have consistently observed that the level of depolarization reached

on the depolarizing peaks of these waves is not the critical determinant of the initiation of firing. In many instances depolarizing peaks exceeded those on which firing occurred without initiation of a spike, suggesting that the relationship between intracellular waves and the spike output may not always be a linear one. The intracellular microelectrode may offer a restricted window on focal membrane potential changes that do not necessarily reflect changes occurring in the vicinity of the axon hillock, where impulse initiation presumably arises. The question of selective internal current paths to the spike triggering zone has been discussed elsewhere (3).

4. The human electroencephalogram in basic behavioral states and task performance; establishment of population means.

To this point, we have considered the EEG in a domain of tissue in terms of contributions from unitary generators. It seems clear that the EEG recorded grossly does indeed closely reflect aspects of cellular behavior within the neuronal population. If, however, we are to generalize across populations of subjects, and to relate the EEG, not only to basic states of consciousness, but also to ever finer grades of performance, we must confront the problem of substantial differences between EEG records from different individuals. Do they, nevertheless, conceal common characteristics not detectable by visual observation?

We have sought to establish by computer analysis the presence of common EEG factors in a population of 50 astronaut candidates, both in relation to task performances and assessment of basic states of sleep and wakefulness (68). To ensure accurate timing in presentation of perceptual and learning tasks, a magnetic tape command system was used, and physiological data was recorded on magnetic tape, together with command signals. The EEG data was subjected to intensive spectral analysis, with calculation of auto- and cross-spectral functions for the 18 bipolar EEG channels from standard electrode placements in all subjects.

After the initial spectral analysis for each subject, the output data was synthesized by an averaging procedure, covering all 50 subjects in the various test situations, and in selected sleep epochs. These averages were made for each scalp region, and are presented as a series of bar graphs, covering the spectrum from 0 to 25 cycles per second. First, an average was prepared of spectral densities at each scalp recording site for all test epochs (Fig. 5, top left) including sitting with eyes closed at rest, eyes closed during 1 per second flash stimuli, during an auditory vigilance task, during visual discriminations at 3 second intervals, and a similar series of more difficult discriminations at 1 second intervals.

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The contours of these "lumped" spectra were then used as the mean for comparison with the spectra for the individual situations. The subsequent graphs in Fig. 5 thus show the variations about the mean established over 12 situations in the top left figure. Spectral densities above the mean at any frequency have bars above the baseline and vice versa. Lengths of these bars are in units of the standard deviation at that frequency, so that relative variation is emphasized.

Such a display clearly separates spectral density distribution for the 50 subjects in the 5 situations shown. Moreover, the distributions for the more difficult visual discriminations (Fig. 5, lower right) exemplify trends that already characterize discriminations made in 3 seconds (Fig. 5, lower middle). Pattern recognition techniques described below clarify differences between records in these two tasks. The method also allows comparison of an individual with the mean for the group, or with his own mean, using a two-color display technique.

5. Pattern recognition techniques applied to spectral parameters in defining attention states.

If, then, a series of group means can be established for the EEG in a variety of states of attention, would it be possible to categorize with equivalent clarity the EEG of a single subject recorded during defined behavioral performances? A discriminant analysis was applied to spectral outputs in 4 subjects covering 5 situations: eyes open at rest, an auditory vigilance task, and the two visual discriminative tasks described above (67). Data from 4 EEG channels was analyzed into 4 frequency bands, corresponding to the classical delta (1.5 to 3.5 c/sec), theta (3.5 to 7.5 c/sec), alpha (7.5 to 12.5 c/sec) and beta (12.5 to 25 c/sec) bands. In each band, measurements were made of the strength of activity in each channel, mean frequency within the band, bandwidth within the band, and the coherence between pairs of channels (Fig. 6).

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This discriminant analysis program first considers all the measurements for all the segments, and selects that parameter which best discriminates segments recorded in different situations. It then reexamines all measurements and chooses the parameter which will add most to the discriminating power of the first measurement, and then continues this iterative process until insufficient improvement is made by adding another parameter. The four variables which best distinguish among the five situations are: left parieto-occipital alpha intensity, the mean frequency of theta band activity in the vertex, the coherence in the theta band between left parieto-occipital and vertex, and coherence in the beta band between vertex and bioccipital leads.

After 15 measurements were selected, the records from individual subjects were correctly classified on 95, 93, 96, and 90 per cent of the tests, compared with only 65 per cent for the group as a whole. It would thus appear that each

subject may have a spatially and numerically characterized EEG "signature," as to which measurements are most effective in distinguishing different situations.

In these first, halting steps in the use of EEG pattern recognition techniques, we nevertheless appear to have within our grasp a tool sufficiently fine to recognize the differences between the EEGs of individuals performing a moderately difficult visual discrimination in 3 seconds and a similar but substantially more difficult task in 1 second. It would seem reasonable to suggest that pursuit of such methods offers an opportunity not only to categorize the complexities of brain wave patterns, but to approach in a far more rigorous frame the underlying subtleties of cerebral system organization in processes of attention (66).

6. Comparison of attention patterns in an astronaut during simulated flight and actual launch into orbit.

The power of the EEG to manifest subtly shifting patterns relating to extreme requirements in focused attention is strikingly demonstrated in our analyses of records from Astronaut F. Borman in an altitude chamber simulation and during the period prior to and following launch in the Gemini GT-7 flight. This data was collected under the supervision of Dr. P.M. Kellaway and Dr. R. Mulsby.

In each study, identical electrode placements and tape recording equipment were used. Electrode placements for the two channels in the simulation and in space flight spanned a wide zone of scalp from vertex to occipital region, with one pair located in the midline, and the other spanning the left parieto-occipital area (47).

(Fig. 7)

In the simulation, samples were analyzed over a 10 minute period characterized by typical alerted patterns, and occasional movement artifacts (epochs 28 to 30 in channel 4, and epoch 29 in channel 5). These contour plots show relatively

low powers at all frequencies above 5 cycles per second, and lack any clear peak in the alpha range around 10 cycles per second. Simple averages of spectral density were prepared for each channel, covering the whole test epoch. The solid line shows a linear plot of spectral densities, and the dashed line a logarithmic plot, over the spectrum from 3 to 30 cycles per second. In both channels, a broad and ill-defined alpha peak at 9 to 13 cycles per second is overshadowed by higher powers in the theta range from 5 to 7 cycles per second. Coherence between the two channels reached significant levels (black areas in right contour plot) only intermittently, and almost solely in the range from 3 to 5 cycles per second. These findings contrast sharply with certain aspects of the flight records.

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The data from the prelaunch period and a substantial part of the first orbit are shown in Fig. 8, with the EEG spectrum plotted on the abscissa, and time on the ordinate. The prelaunch period was characterized by increased amounts of theta rhythms than was seen in baseline records. At one minute before lift off, there was a further increment of theta activity and in the higher frequencies in the alpha and beta bands. This may be interpreted as relating to strongly focused attention and orienting responses in this novel situation. The power density of the EEG was augmented by a factor of 10 over many frequencies before and following launch, indicating a strong EEG "arousal reaction." Thereafter, there was a slow decline in these augmented densities, with recurrent epochs of higher powers in the higher frequency bands above 10 cycles per second in the first half hour of flight.

Coherence measurements between the two channels (right hand figure in each row) showed striking differences from the simulation baseline. Coherence grew to significant levels (black areas) at progressively higher frequencies in the range from 3 to 9 cycles per second up to the moment of launch. Thereafter it declined to non-significant levels at most frequencies for the next 30 minutes.



This was followed by an enormous rebound (in the lower figure) to extremely high levels in the range from 3 to 11 cycles per second for the next 40 minutes, and a progressive decline thereafter. These high coherence levels were not seen in any of the ground based recordings, nor in any subsequent part of the flight records.

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It is rarely indeed that one has an opportunity to secure such data in circumstances requiring highly focused attention under conditions of severe environmental stress. The findings suggest that coordinated activity between large masses of cortical tissue, as measured by the coherence between them, may depend critically on anticipatory or recapitulatory aspects of a novel and hazardous experience.

#### 7. Electrophysiological studies of arousal, orientation and discrimination.

From the broad window on patterns of brain electrical activity available from scalp records, we may return to finer patterns to be discerned in cortical and subcortical structures as concomitants of alerting, orienting and discriminate responses. The development of conditional firing patterns in cerebral neurons will be briefly reviewed prior to discussion of EEG processes also related to establishment and maintenance of conditional phenomena. These topics have been discussed in detail elsewhere (3).

##### a. Unit firing patterns in conditional responses.

Polymodal input to individual cells in brainstem and diencephalic reticular structures (49,60) has suggested that "temporary connections" may be established via these cells. Despite great variability of responses in cortical structures, meaningful patterns have also been detected at this level (42,51,71). The importance of reticular structures in conditioning has been established in both recording and lesion experiments (22,25,37). Olds and Olds (51) have emphasized the relative ease of establishment of conditional behavior in paleocortical and subcortical units, by comparison with cortical units.

Medial thalamic units can be repeatedly extinguished and retrained during classical conditioning. Their behavior during repeated conditioning and extinction emphasizes the plasticity of responsiveness of single cells in such a paradigm, with gradual appearance of firing patterns which might be the converse of those initially elicited (45). Extinction tests following each conditioning also exhibited progressive rebound phenomena, so that over a period of several hours it was possible to detect a series of gradual changes that increased in magnitude, as well as showing qualitative differences from those in the first extinction trial (Fig. 9).

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The "training" situation used by Kamikawa et al. (45) involved pairing of a light flash as a conditional stimulus with an unconditional shock train to the sciatic nerve. The CS-US interval ranged from 300 to 800 msec. Tests with intervals shorter than 300 msec failed to elicit a conditional response. Training trials were given once every 10 sec. A change in firing in the CS-US interval characteristic of a conditional response required a minimum of about 50 trials, presented over a period of about 20 minutes.

These findings offer some possibility of an understanding of processes that might underlie lasting structural changes associated with a memory trace. The required minimal CS-US interval of the order of 300 msec suggests a time scale comparable with electrophysiological events at the neuronal membrane such as the prolonged inhibitory postsynaptic potentials seen in cortical neurons (16) lasting up to several hundred milliseconds, and without an equivalently persistent counterpart in spinal motoneurons; or with wave processes in cortical neurons, which at this time are less definitely related to synaptic potentials (5). On the other hand, the requirement that training trials have a time course of 20 minutes to generate a conditional response suggests a much slower process, perhaps the synthesis of a macromolecule,

protein in nature, and located at the neuronal membrane, where it might directly influence the excitability of the cell by synaptic volleys.

b. EEG correlates of orientation and discrimination.

The characteristics of spontaneous rhythm changes in classical conditioning have been reviewed in detail elsewhere (3). These studies of the activated EEG in classical conditioning have contributed to the view that the desynchronizing response represents a stereotype that can scarcely be pursued further in finer analysis of correlates with behavioral responsiveness and conditioning. Yet it has long been recognized (41) that certain manipulations of a classical conditioning procedure leads to trains of synchronous slow waves. This was observed where the CS-US interval was prolonged, or in cortical areas surrounding a focal activated response, and was attributed by Gastaut (36) to "internal inhibition" in the Pavlovian sense.

Recognition that a synchronous, rather than activated, pattern of cortical activity accompanies certain aspects of classical conditioning, has led to a finer analysis of distribution of wave activity in cortical and subcortical structures regularly occurring in certain operant performances, and to extensive computer analysis of their patterns (4,11, 12, 56, 58, 66).

Much attention has been directed to allocortical structures of the amygdaloid and especially the hippocampal systems in deposition of the memory trace (1,14,17,38). The great antiquity of the hippocampal system in evolution of the brain, and the essential stability of its basic structure in the face of immense evolutionary changes in the remainder of the cerebral mantle are in themselves a challenge to an understanding of its functional role. Despite strongly suggestive evidence for its participation in essential processes of memory even in simple brains, persisting difficulties in such an easy interpretation demand a cautious attitude. Thus, the memory trace may be laid down

outside the hippocampal system (55), but integrity of its interrelations with these seemingly unrelated cortical and subcortical regions may be vital to the appropriate recall of previously learned discriminative habits (13). In consideration of sometimes incompatible and even contradictory findings from a variety of lesion studies, Drachman and Ommaya (26) have concluded that the essential defects after hippocampal lesions involve impairment of acquisition and loss of retention, rather than impaired short-term memory.

Our studies in the hippocampal system in the course of learning a discriminative task have sought evidence of altered electrical patterns closely correlated with acquisition of a learned task, and more fundamentally, whether such modifications might suggest anything about the essential processes of storage in cerebral tissue (4,11,12,13,58,65). The neuron may be considered in terms of its ability to sense complex spatiotemporal patterns of waves induced at its surface. Such a frame of functional organization would assist in defining the possible uniqueness of integrative processes in cortical systems, characterized by dendritic overlap in a palisade arrangement of cells as described above, and with wave phenomena as a concomitant of electrotonic processes, which appear largely localized in dendritic structures.

We have extensively studied hippocampal EEG activity in the cat in the course of acquisition of a visual discriminative performance, in a modified T-maze, with approach to a concealed food reward on the basis of a visual cue. Whereas alerted behavior was accompanied by a wide spectrum of activity at 4 to 7 cycles per second, with a 4 cycles per second dominant, the period of discriminative performance was characterized by a very regular burst of "theta" waves at an essentially single frequency around 5.5 cycles per second in the dorsal hippocampus, and in the entorhinal area of the pyriform cortex.

Concurrently, less regular and less constant rhythmic processes were frequently noted in subcortical structures including midbrain reticular formation and subthalamus. The latter are under continuing investigation (27).

i) Assessment by computed averages of hippocampal and midbrain reticular EEGs during task acquisition and after cue reversal in the cat.

When discriminative ability was still at chance levels, but a relatively stable response pattern and approach latency were already established, computed averages of hippocampal EEGs during 30 or 40 daily trials showed some rhythmicity at 5 cycles per second. In the course of subsequent training, there was frequently a decline in rhythmicity of the average at performance levels between 80 and 90 per cent. At performance levels around 100 per cent, a greater degree of regularity was noted than at any previous stage of training (Fig.10). The transient decline in rhythmicity at mid-training did not relate to a decline in regularity of the 5 cycles per second burst in individual records. It thus appears to have resulted from either a loss of phase-locking of these bursts to the onset of the situational presentation at mid-training, or, possible, to the appearance of significant degrees of frequency modulation on the 5 cycles per second bursts, as detected by sensitive digital filtering techniques (11). By contrast with subcortical structures, such as the midbrain reticular formation, the regular components of the hippocampal EEG persisted into substantial overtraining, although the duration of the regular burst was often abbreviated.

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On the first day after cue reversal, averages of hippocampal activity were extremely regular at 5.5 cycles per second, higher in amplitude than before cue reversal, and sustaining throughout the approach. The increase in averaged output apparently resulted from diminished scatter in phase patterns in consecutive performances, rather than from increased amplitude in the individual bursts.

In ensuing training days with performances from 50 to 75 per cent, there was a progressive decline in amplitude and regularity of the computed hippocampal average. On attainment of a performance level around 90 per cent in the new paradigm, a highly rhythmic average again appeared. Repeated cue reversals with retraining to high performance levels, or substantial overtraining in a particular paradigm, led only to shortening of the length of the regular average.

By contrast, subcortical structures, such as the midbrain reticular formation, showed after each cue reversal a decline and then a gradual reestablishment of a regular average at high performance levels. Beyond the fifth or sixth cue reversal over a six month period, a sophistication in the situation appeared, with a rapid rise in performance in the first few training days after cue reversal. Reticular records did not regain a rhythmicity comparable with that in earlier tests, even at performance levels over 95 per cent. It may be surmised that information essential for discrimination may have reached minimal proportions, and that appropriate behavioral performance may occur with little more than fleeting attention to behavioral cues. A high scatter might once again appear in phase patterns of successive records, but subtly different from the irregular patterns in early training.

ii) Comparison of EEG patterns in orienting and discriminative behavior.

The mammalian response to a sudden stimulus runs a gamut from the "startle response," with arrest of ongoing behavior, through various investigative reactions, to an almost infinite variety of complex coordinated motor patterns, constituting "fight or flight" responses (21). It is in the second category that we may group the behavioral components of the orienting reflex, as first characterized by Pavlov (53). Its uniqueness rests on certain "principles" in the intimate behavior of its component reflexes, including their non-specificity with respect to both quality and intensity of the stimulus, and the selectivity of various properties of the stimulus with repeated presentation (62,63).

Although a specific relationship has been postulated between hippocampal theta wave trains and orienting behavior (39), the exquisite plasticity of hippocampal theta rhythms in changing behavioral states, including the appearance of bursts of waves in a narrow spectral range during performance of a visual discriminative task, have suggested more subtle and specific relations to discriminative functions and judgment capability (2,4,13,58).

Radulovacki and Adey (58) found it possible to distinguish hippocampal EEG activity in three basic states in the cat; in alert but non-performing animals, in the course of discriminative performances, and during orienting behavior. Alert but non-performing animals exhibited a wide spectrum of "theta" waves in the range 3 to 7 cycles per second on first introduction into the test situation, without overt aspects of orienting behavior. During T-box discriminative performance, theta waves regularized at 5 to 6 cycles per second, as described above. Computed averages in orienting trials, given in the same numbers on each test day and randomly interspersed with discriminative trials, showed slower and less regular averages at 4 to 5 cycles per second (Fig.11).

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These studies indicate that in the cat, hippocampal wave trains relate in clear and specifiable ways to the performance of a discriminative task, and in different, but equally recognizable patterns, to aspects of orienting behavior. Collectively, these studies in hippocampal, sensory cortical, midbrain and subthalamic areas have suggested that the deposition of a "memory trace" in extrahippocampal systems may depend on such wave trains, and subsequent recall on the stochastic reestablishment of similar patterns (11).

Although no causal relationships can be established at this time between the decision making process and a particular EEG pattern, the detection of differences in wave patterns in correct and incorrect responses (12,13), using cross-correlation and cross-spectral techniques, has emphasized the strong

possibility of a stochastic mode of operation in the handling of information on the basis of a wave process. Such a scheme would envisage the excitability of the individual neuron as depending not only on its previous experience of complex spatio-temporal patterns of waves, but would also suggest that the effectiveness of any subsequent wave pattern in eliciting neuronal firing might depend on its multivariate relationship to an "optimal" wave pattern, capable of inducing firing of that neuron at its lowest threshold.

#### 8. Impedance changes in alerting, orienting and discrimination in the cat.

In a series of studies, we have measured the impedance of focal volumes of cerebral tissue with chronically implanted coaxial electrodes at 1000 cycles per second (7,8,9,10,57). The technique has been described in detail elsewhere (44) and uses a current density of  $10^{-13}$  A per  $\mu^2$  of electrode surface, with a differential sensitivity one hundredth of that current level. The probable pathways for these low level measuring currents lie through the extracellular fluid and neuroglial cells, since both may be presumed to offer paths preferred over the substantially higher impedance route through neuronal membranes.

Electrical impedance was measured in the hippocampus, amygdala and midbrain reticular formation during alerting, orienting and discriminative performances (10). The impedance of small volumes of cerebral tissue changed differentially at different sites in the course of this repertoire of alerting and learned responses. The magnitude of these impedance responses increased as levels of performance rose progressively above chance. They were susceptible to cue reversal and subsequent retraining. Variability as indicated by standard error of the mean, was large in records from initial training trials, decreased progressively with training, reverted to wider levels on cue reversal, and decreased once more with retraining (Fig. 12).

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Fig. 12  
here



In the fully trained animal, computed averages of hippocampal impedance decreased by as much as 8 per cent of baseline during visual discrimination, whereas alerting and orienting responses immediately preceeding were not accompanied by comparable impedance changes. Similar measurements in the rostral midbrain reticular formation showed small responses during orientation and discrimination, and less constantly during alerting responses. The amygdala exhibited consistent responses only in the alerting epoch.

9. The nature of the impedance response: the role of neuronal elements and the influence of divalent cations on extracellular macromolecules.

From the foregoing, these impedance responses appear to relate to changes in intrinsic characteristics of cerebral tissue. Our studies have indicated that regional differences in impedance responses occur with shifts in carbon dioxide levels induced by hypercapnea or hyperventilation, and that they do not arise in simple relationship to alterations in blood pressure, cerebral blood flow or brain temperature (9,57).

It is generally agreed that extracellular space offers a high conductance pathway, but its precise relationship to the observed impedance requires consideration of its extent in cerebral tissue, and its content of macromolecular and ionic material, as discussed above. If it is the site of the impedance changes described here, then we must seek evidence of a temporary and presumably reversible movement of ions into it, a movement presumably initiated in neuronal elements, but capable of modulation by neuroglial cells, or of influencing neuroglia.

To test the role of neuronal elements, retrograde degeneration was induced in the lateral geniculate body by unilateral resection of the visual cortex. This led to loss of about 80 per cent of lateral geniculate neurons, and was followed by perturbations in geniculate impedance baselines from 10 to 30 days

postoperatively. Subsequently, impedance responses to alcohol and to a cyclohexamine drug were reduced to about 20 per cent of those in the intact nucleus, thus indicating a requirement for an intact neuronal population in the normal manifestations of impedance responses.

The findings suggest that the impedance responses are mediated through perineuronal compartments, with modulation by either neuronal or neuroglial elements of conductance in an intercellular fluid containing a matrix of macromolecules. The extent to which changes in neuroglial membrane resistance might contribute directly remains uncertain (50).

The susceptibility of intercellular macromolecules to divalent cations, such as calcium, has led us to investigate the effects of injection of small quantities of calcium salts into the lateral ventricle in the cat, while recording with chronically implanted coaxial electrodes the impedance in periventricular structures, including hippocampus, caudate nucleus and amygdala (69).

Injections of calcium chloride solution (40 to 60 microequivalents in 0.1 ml) were made, preceded by control injections of normal saline. No changes followed the control injections. A sharp decline occurred in both resistive and reactive components, beginning 15 to 30 minutes after injection of 40 to 180 microequivalents of calcium solution, in the structures named above, bounding the lateral ventricle (Fig. 13).

Insert  
Fig. 13  
here

Impedance readings were shifted from baseline values by as much as 25 per cent for periods that exceeded 36 hours in some cases. Changes were largest and earliest in structures closest to the tip of the cannula, and were delayed by 30 to 50 minutes in reaching peak values in symmetrically placed leads in the opposite half of the brain. Maximum shifts occurred within two hours of injection. The intraventricular injection was regularly followed by a topographically determined sequence of impedance changes, consistent with diffusion

from the injection site via the cerebrospinal fluid. On the other hand, direct injection of up to 120 microequivalents into periventricular structures was without comparable effects, except at electrodes immediately adjacent to the injection site. The onset of these impedance shifts in hippocampus and amygdala was accompanied by seizure-like discharges, but the impedance shifts were prolonged many hours beyond cessation of gross EEG abnormalities.

Thus the evidence is consistent with the view that cerebral impedance changes accompanying physiological responses may arise in perineuronal fluid with a substantial macromolecular content, and that calcium ions may modulate perineuronal conductivity, as well as fluxes of sodium and potassium across the neuronal membrane. In such a frame, the disclosure of impedance changes in cerebral tissue in the course of alerting, orienting and discriminative responses, their selective regional distribution, and dependence on levels of learning, all invite consideration of the role of perineuronal elements in aspects of transaction and storage of information in brain tissue.

#### Summary

This review has considered the gamut of neural organization, ranging from subcellular events in the genesis of intracellular waves, to the patterns in scalp EEG records characterizing a population of human subjects in states of focused attention and visual discrimination. A tricompartmental model of cerebral tissue is described, with neuronal, neuroglial and extracellular divisions. The role of macromolecular systems at the neuronal surface and in the intercellular fluid is considered. Evidence is presented that mucoproteins and mucopolysaccharides may be responsible for net fixed charges at the cell surface, and may thus play a role in ionic fluxes across the membrane. Divalent

cations, such as calcium, may modify these macromolecular configurations. Impedance changes in cerebral tissue accompanying alerting, orienting and discriminative responses are described, with emphasis on their regional distribution, and relationship to levels of learning. Their possible origin in altered conductance in extraneuronal compartments is discussed. Genesis of the electroencephalogram in a population of neuronal wave generators is reviewed, and evidence presented that these generators are non-linearly related, with the EEG arising as a normal distribution from the combined activity of such non-linearly related neuronal generators.

#### Acknowledgments

The studies from our laboratory presented in this paper have involved the dedicated efforts of my many colleagues in the Space Biology Laboratory, to whom I offer my sincere thanks. These studies were supported by Grants NB-01883 and MH-03708 from the National Institutes of Health, Contract AF(49) 638-1387 with United States Air Force Office of Scientific Research, Contract NONR 233(91) with the Office of Naval Research, and Contracts NSG 237-62, NSG 502, NSG 505 and NSG 1970 with the National Aeronautics and Space Administration.

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### LEGENDS TO FIGURES

- Fig. 1. Induction of movement in a body having net fixed surface charge in the presence of a non-uniform electric field. (From Elul, 1966b).
- Fig. 2. Typical examples of cortical surface (top trace) and intracellular (lower trace) records in the same domain of tissue. Initiation of action potentials in the intracellular records occurs on the depolarizing phase of the concurrent wave process, but not necessarily on the largest waves. Records A and B during sleep show slow waves in both extracellular and intracellular traces, whereas the alerted record (C) is faster in both. Calibrations for EEG channel, 50 microvolts; for intracellular records, 50 millivolts. (From Elul, 1965).
- Fig. 3. Auto-spectral density distributions for simultaneous intracellular and surface EEG records from the same region of suprasylvian cortex in the cat. Comparisons are for two different neurons, and show similarities in spectral contours between surface and cellular records. (From Elul, 1965).
- Fig. 4. Plot of coherence over a 500 second epoch between intracellular wave records and EEG from cortical surface in same domain of tissue. Coherence levels are below statistically significant levels at all frequencies for the major part of the analysis epoch, and the incidence of significant levels of coherence (shown in black) remains around chance levels throughout the analysis. The findings are interpreted as indicating origins of the EEG in a population of independent neuronal generators (see text). (From Elul, 1965).

- Fig. 5. Averaged spectral densities over the range 0 to 25 cycles per second for a population of 50 subjects, with each spectrum presented as a series of bars at 1 cycle per second intervals, and placed at the appropriate location on the scalp. The top left figure is an average for all subjects across 12 situations (see text). The contour of this average was then used as the mean against which to measure deviations in the succeeding five situations, with powers above the mean at any frequency shown as a bar above the baseline and vice versa. Calibrations for average over 12 situations in microvolts squared per second per cycle; for the separate situations, in standard deviations. (From Walter, Rhodes, Kado and Adey, 1966).
- Fig. 6. Pattern recognition techniques applied to spectral outputs from 4 subjects, separately and jointly, with development of a matrix display of automated classifications for five situations: EC-R, eyes closed resting; EO-R, eyes open resting; EC-T, eyes closed while performing an auditory vigilance task; EO-T-3, performing moderately difficult visual discriminations in 3 seconds; EO-T-1, performing difficult visual discriminations in 1 second. (From Walter, Rhodes and Adey, 1966).
- Fig. 7. Analysis of EEG records from altitude chamber Gemini flight simulation. Electrode placements, amplifiers and recording equipment were identical with actual flight systems. Left and middle contour plots show auto-spectral densities in the two EEG channels. Right hand plot shows coherence between them. Averaged spectral densities for each channel were prepared from more than 40 epochs, each 20 seconds in duration. (Solid line in lower traces,

linear plot; dashed line, logarithmic plot). Coherence between EEG channels (right contour plot) reaches statistically significant levels only sporadically (black zones), mainly at frequencies between 3 and 5 cycles per second.

Fig. 8. Contour maps of EEG data from F. Boman in Gemini Flight GT-7. Prelaunch and launch epochs are shown in the top row of maps. Auto-spectral densities in the theta range were augmented in the two EEG channels (CPEEG4 and CPEEG5) prelaunch, with great exaltation of many EEG frequencies immediately before and during lift-off. Coherence between the channels (CPEEG5/CPEEG5) became significant at progressively higher frequencies in the 15 minutes before launch, but declined sharply at lift-off for the first 5 minutes of flight. Thereafter the coherence in the range from 3 to 11 cycles showed a gradual return at progressively higher frequencies, and in continuation of the first orbit (B), attained very high values across the spectrum from 3 to 11 cycles, in a fashion not seen in any control records on the ground, nor in subsequent flight records. Calibrations in auto-spectral contours are in microvolts squared per second per cycle. Shaded contours are 100-300  $\mu\text{V}^2/\text{sec}/\text{cycle}$ , horizontal shading; 300-1000, vertical shading; over 1000, solid black. In the coherence plots, values above 0.7 (statistically significant level) are in black.

Fig. 9. Development of an inhibitory conditional response in an habenular unit. Each dot represents a unit discharge, and each horizontal row of dots, a single trial. Trials are grouped according to stimulus conditions. A: sciatic nerve stimulation only (US) as control; B: flash only (CS) control; C: flash and sciatic paired

in first sequence of training trials. Left vertical line indicates time of CS presentation. Vertical line marked "sciatic" indicates time of onset of US. (From Kamikawa, McIlwain and Adey, 1964).

Fig. 10. Representative EEG records with computed averages from 20 pairs of hippocampal traces at mid-training after first cue reversal (left). Note irregular character of averages. Later in training, rhythmic average appeared (right) and was sustained into overtraining. (From Adey and Walter, 1963).

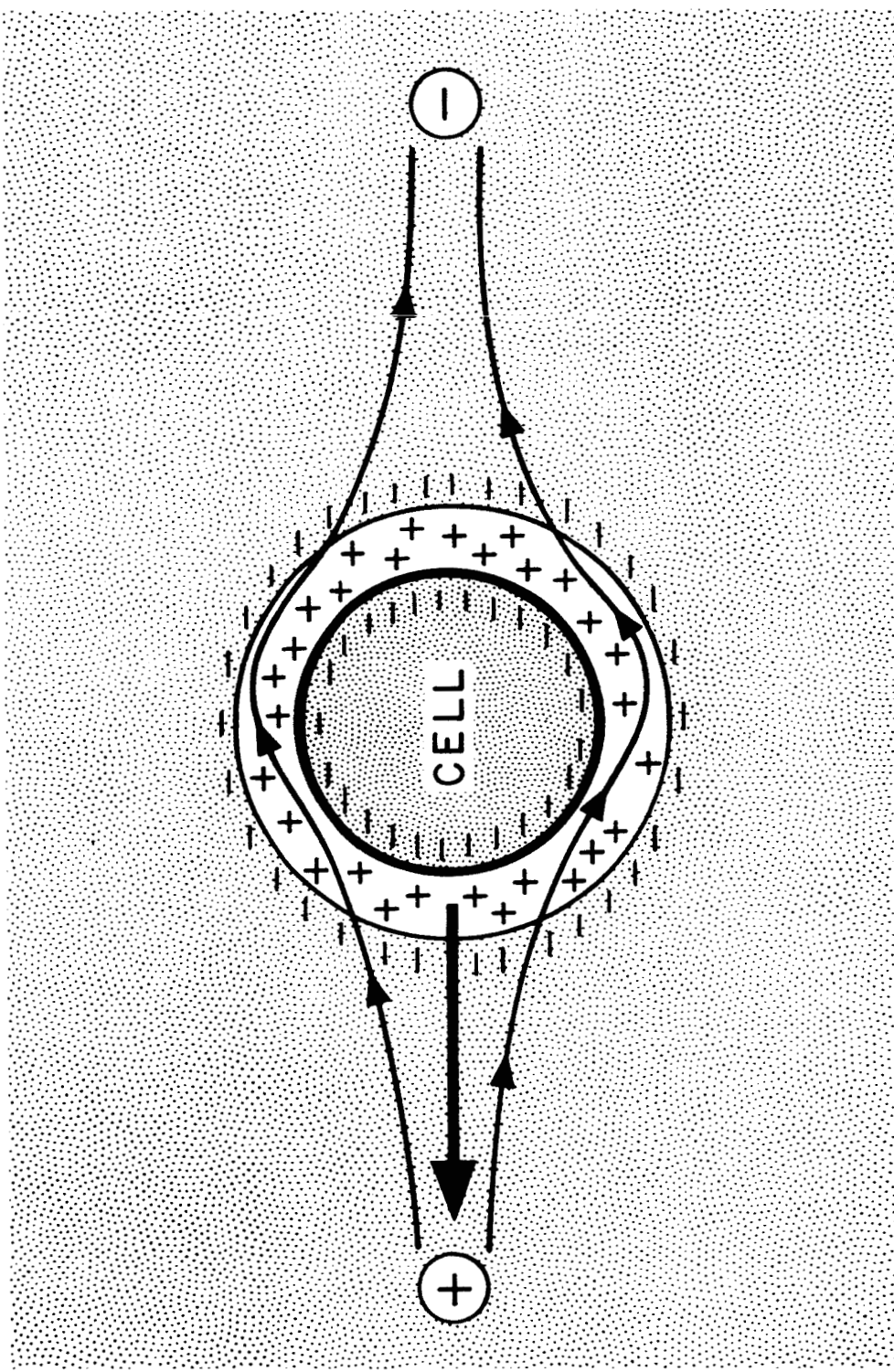
Fig. 11. Effects of introduction of orienting trials (daily  $w=40$ ) into training schedules of a cat already at a high level in discriminative task performance. Computed averages during discrimination (A) showed high amplitude waves at 6 cycles per second. Randomly interspersed orienting trials (B) exhibited a lower amplitude to 4 to 5 cycles per second rhythm in later parts of analysis epoch. (From Radulovacki and Adey, 1965).

Fig. 12 Calculations of means and variability in hippocampal impedance over 5-day periods at various levels of training, with successive presentations of alerting, orienting and discriminative stimuli. In each graph, middle trace indicates mean, with upper and lower traces showing one standard deviation from the mean. Calibrations indicate 50 picofarads, with mean baseline at 11.1 kilopicofarads throughout the training maneuvers; and 100 ohms, against a mean baseline of 16.0 kilohms for the same period. Variability was low at 100 per cent performance (A), increased substantially immediately after cue reversal (B), but decreased again after retraining (C). (From Adey, Kado, McIlwain and Walter, 1966).

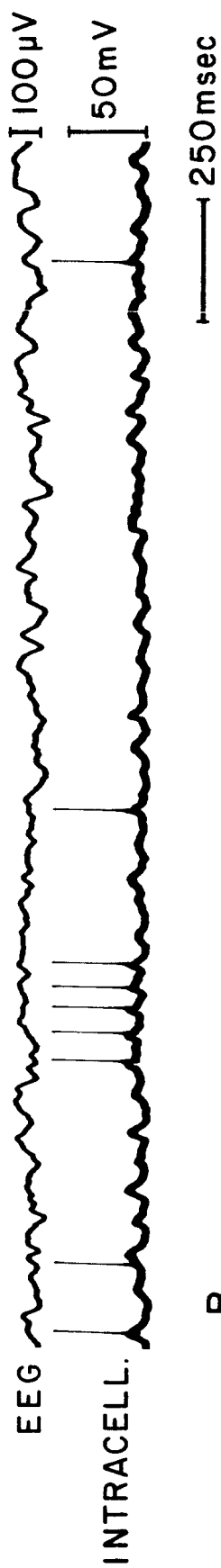


Fig. 13. Impedance measurements in left and right caudate nuclei, with chronically implanted coaxial electrodes. Control injection of 0.1 ml normal saline at 50 minutes was followed by 3 injections of calcium chloride (each 60 microequivalents in 0.1 ml) at 110, 175 and 210 minutes. Resistive (solid line) and reactive (dashed line) readings are shown for both structures. Ordinate scales: resistance in Kilohms ( $K\Omega$ ) and capacitance in Kilopicofarads (Kpf). (From Wang, Tarby, Kado and Adey, 1966).

# CELL MOVEMENT INDUCED BY ELECTRIC CURRENT



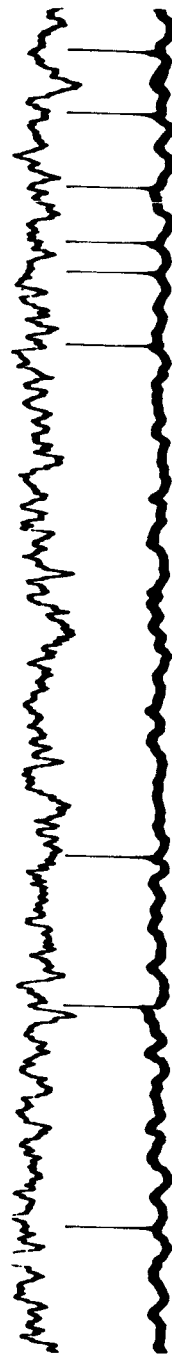
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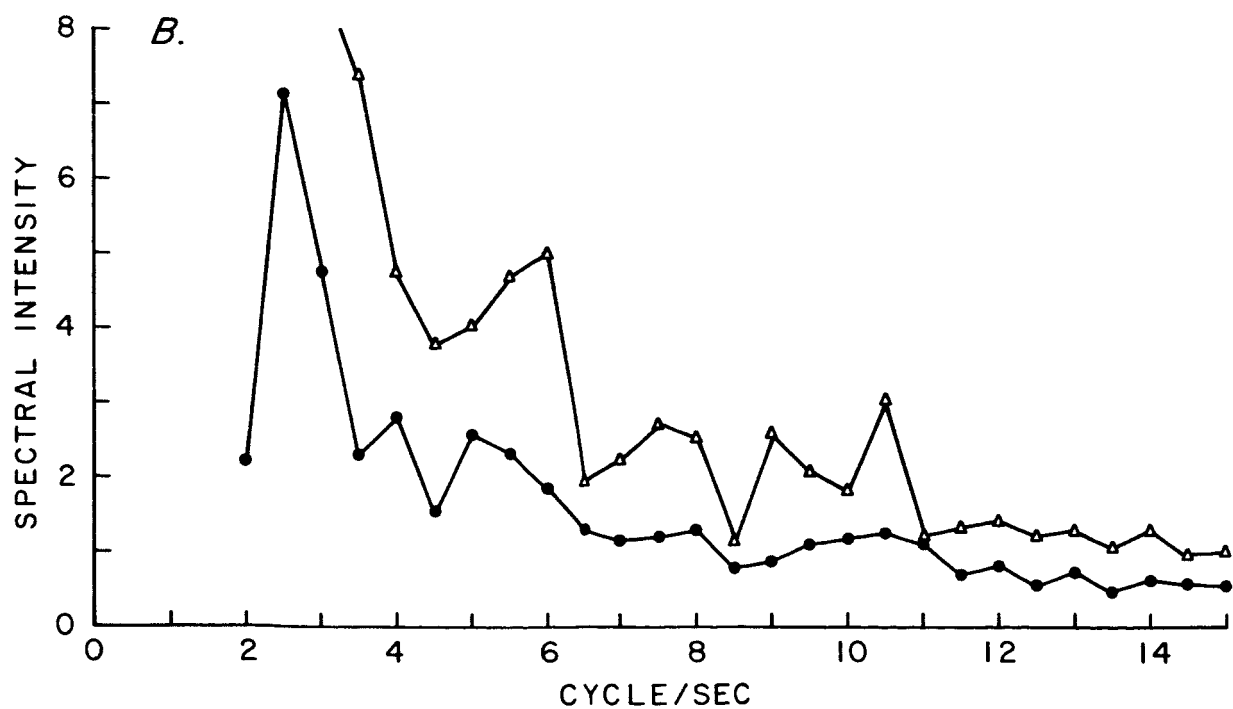
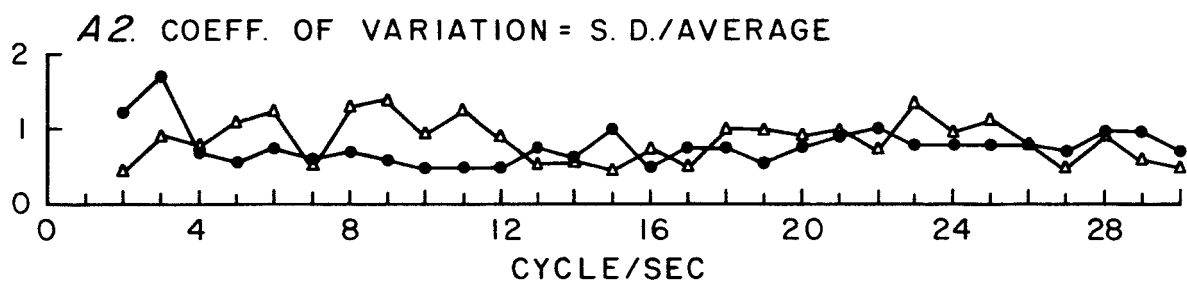
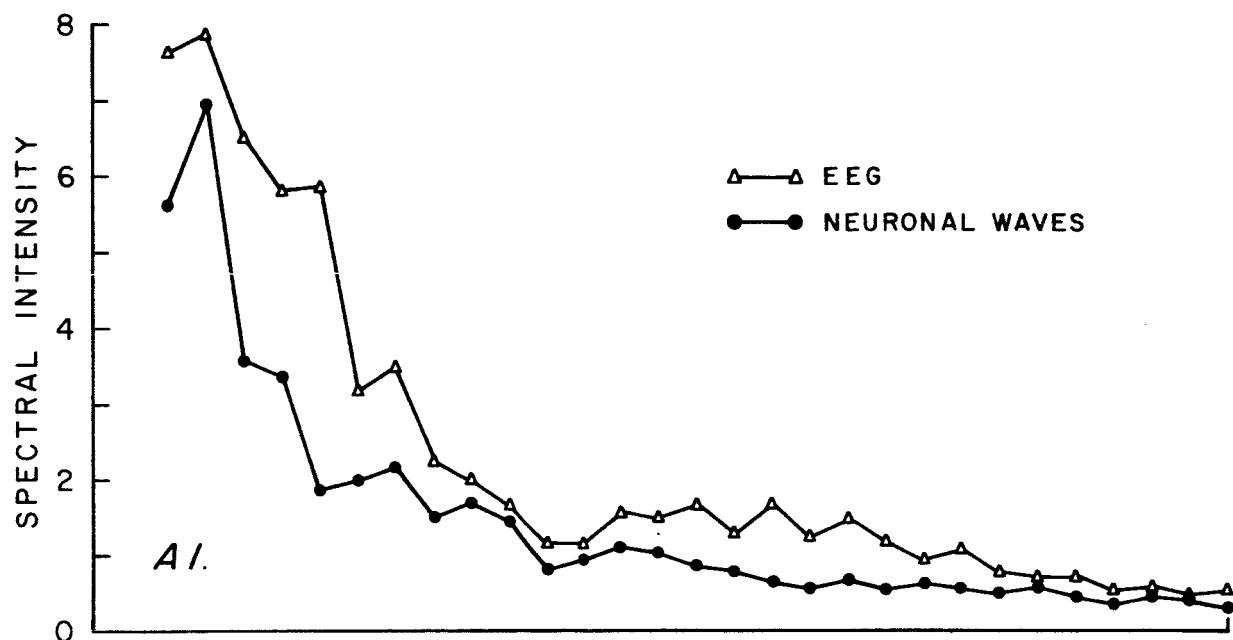
B.

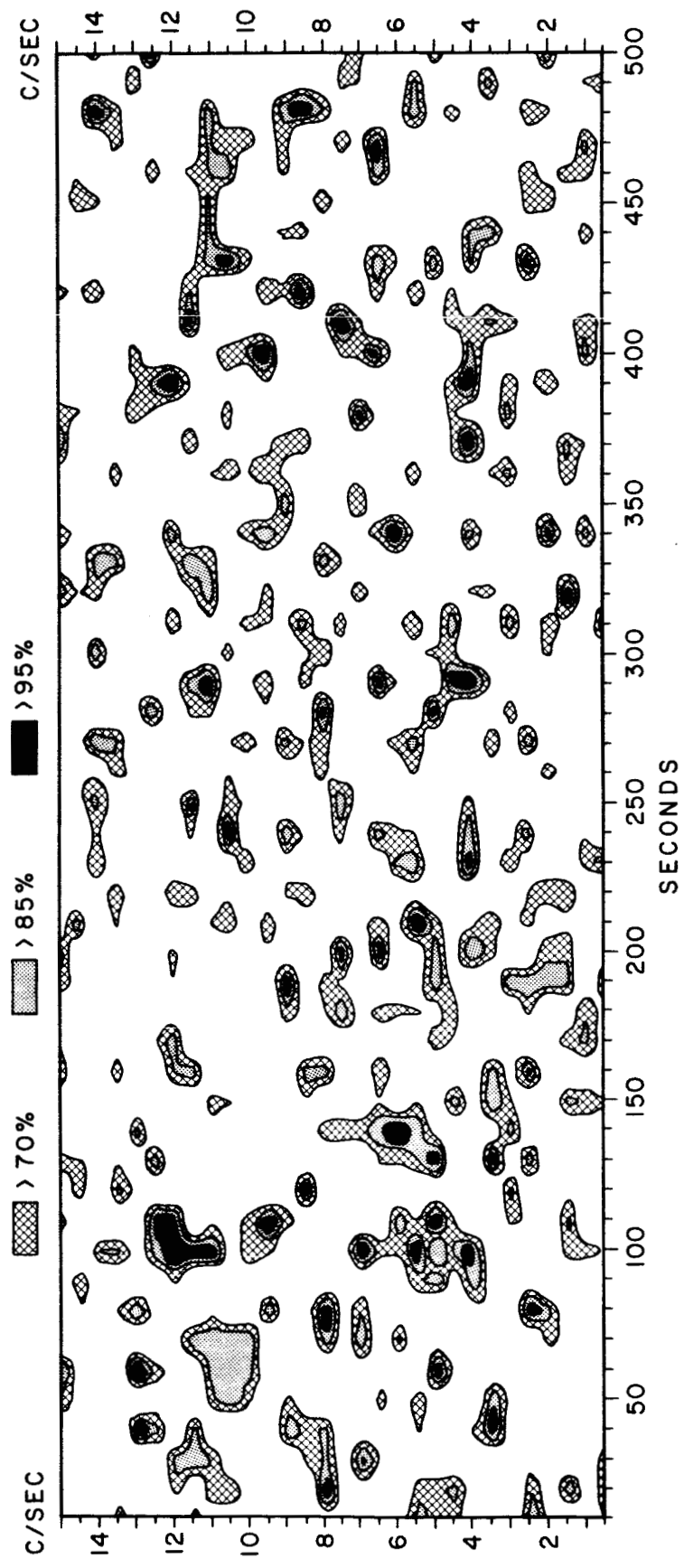


C.



# POWER SPECTRA OF INTRACELLULAR ACTIVITY AND EEG

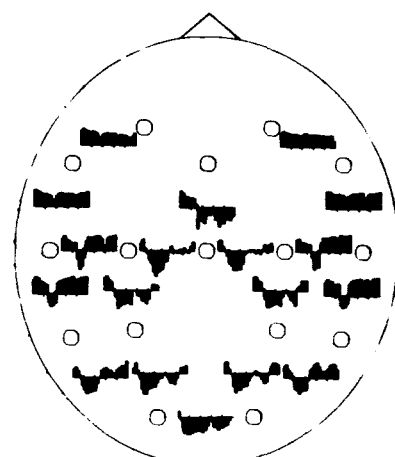
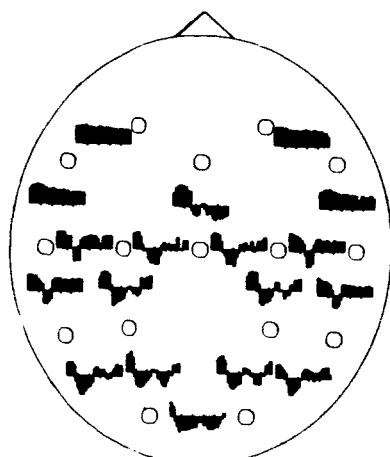
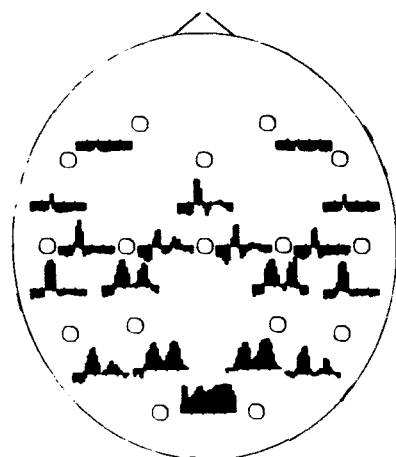
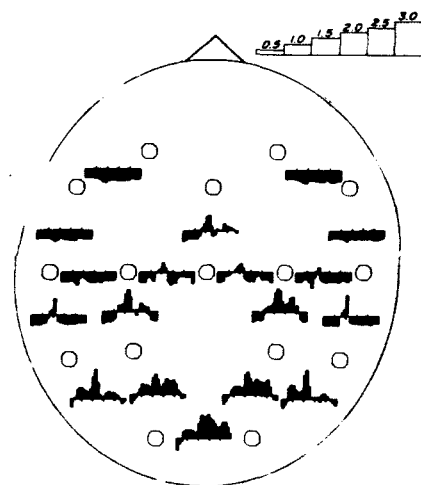
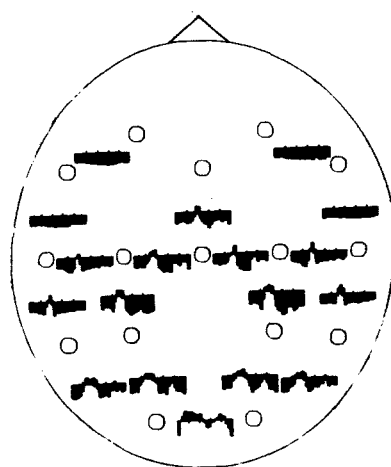
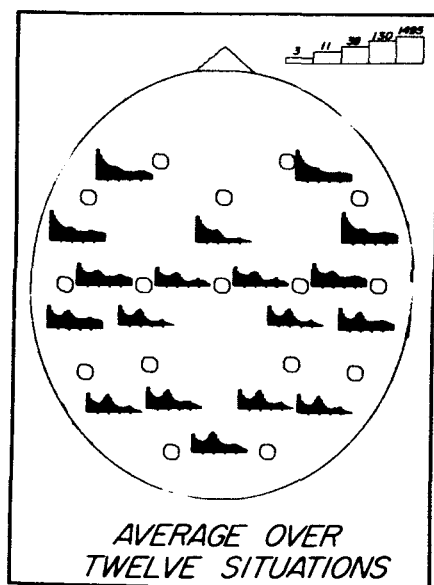




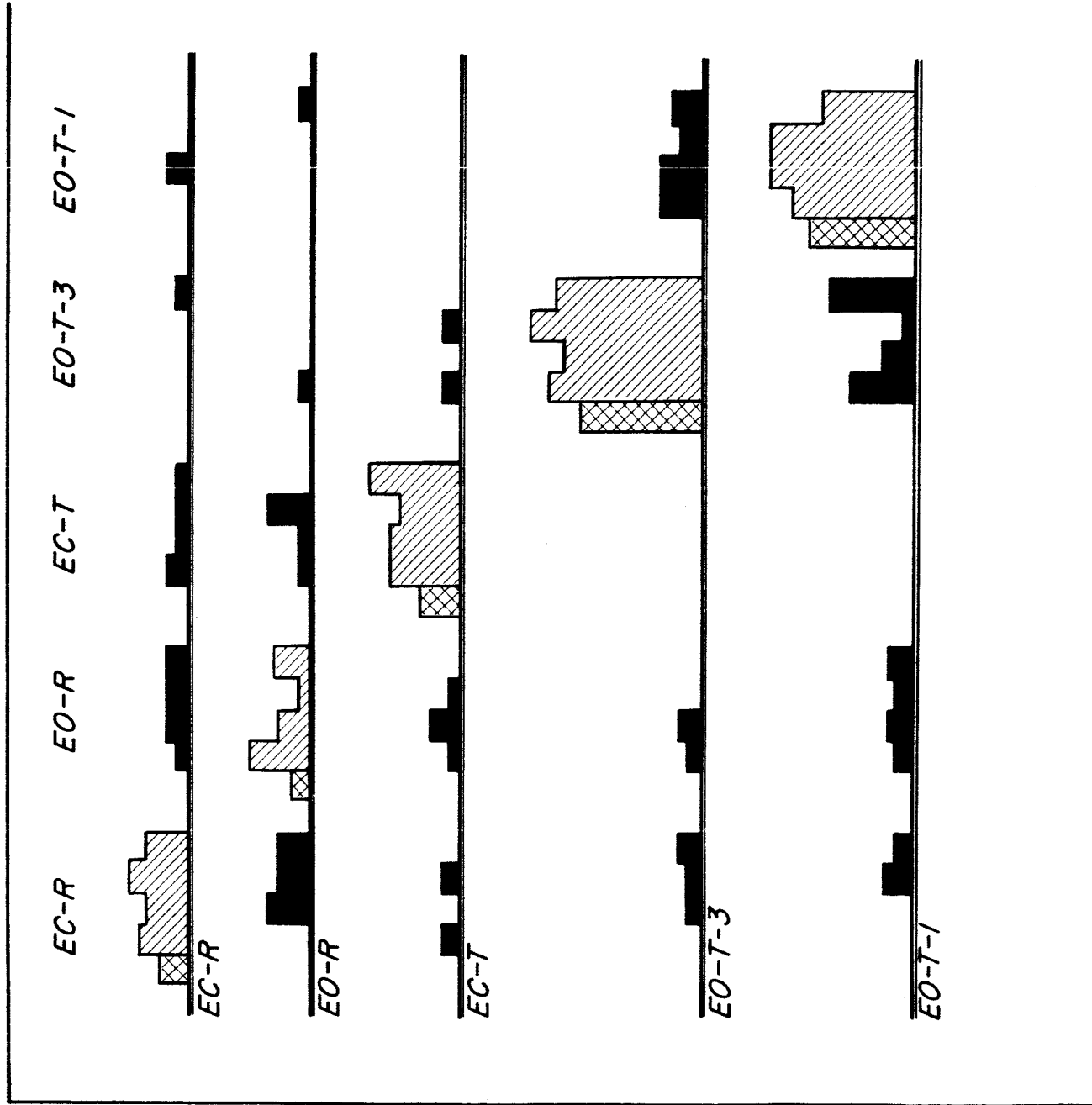
COHERENCE (LINEAR PREDICTABILITY RELATIONSHIP)  
BETWEEN NEURONAL WAVES AND EEG

# RESPONSES OF ELECTROENCEPHALOGRAPH TO DIFFERING SITUATIONS

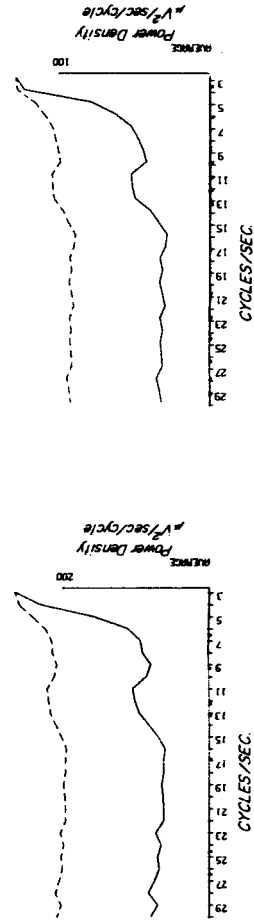
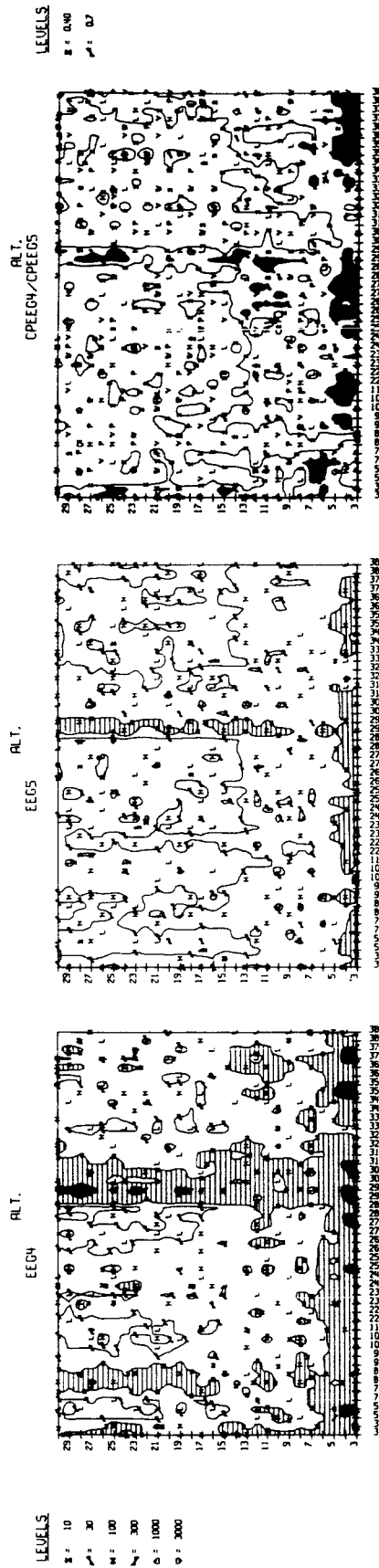
TOPO-SPECTROGRAPHIC VARIATIONS OF  
AVERAGES OVER FIFTY ASTRONAUT CANDIDATES



# AUTOMATIC CLASSIFICATION BY BEST 4 MEASUREMENTS



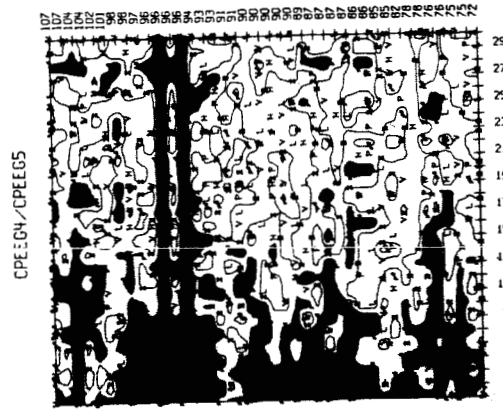
COHERENCE BETWEEN CHANNELS 4 and 5





### A. PRELAUNCH-ORBITAL INJECTION

19 PWD  
 71 THE TA  
 68 ALPHA PRA  
 68 BETA PRA  
 68 THE TA  
 64 THE TA  
 63 DELTA THE TA  
 61 THE TA  
 59 THE TA  
 58 THE TA  
 56 BETA  
 56 BIG SLOW THE TA  
 55 SLOW THE TA  
 55 LOW ALPHA SURVAYS  
 50 DELTA  
 50 BETA THE TA - SURVAYS  
 49 DELTA THE TA  
 47 HIGH THE TA - SURVAYS  
 45 MUCH LOW THE TA  
 43 THE TA  
 41 THE TA AND BETA  
 39 THE TA  
 37 BIG SURVAYS  
 36 THE TA - LOW ALPHA THE TA  
 35 THE TA - LOW ALPHA THE TA  
 34 THE TA AND BETA AND VIBRATION  
 33 THE TA AND BETA AND VIBRATION  
 31 START  
 30 LOW ALPHA THE TA  
 29 PINKED THE TA AND BETA  
 28 THE TA  
 28 SOME THE TA  
 27 SOME COLLECTION  
 26 APPARENTLY WITH EFFECT  
 25 APPARENT SLOW WAVES  
 24 QUIET LEG  
 23 THE TA  
 22 THE TA  
 21 THE TA  
 20 THE TA  
 19 THE TA  
 18 THE TA  
 17 THE TA  
 16 THE TA  
 15 THE TA  
 14 THE TA  
 13 THE TA  
 12 THE TA  
 11 THE TA  
 10 THE TA  
 9 THE TA  
 8 THE TA  
 7 THE TA  
 6 THE TA  
 5 THE TA  
 4 THE TA  
 3 THE TA  
 2 THE TA  
 1 THE TA

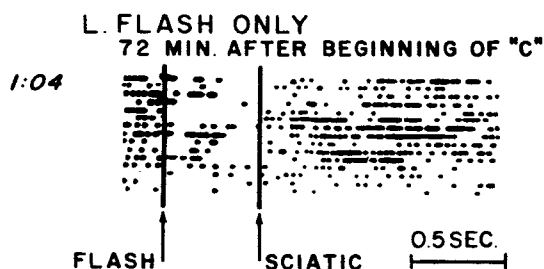
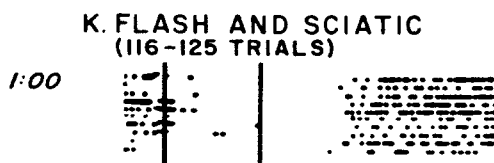
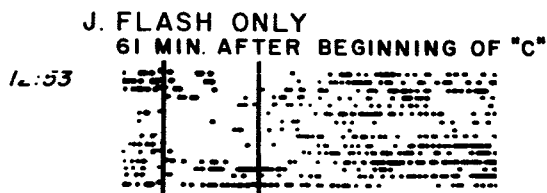
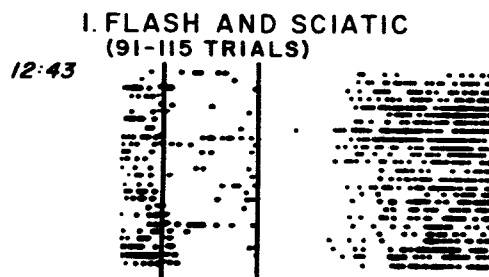
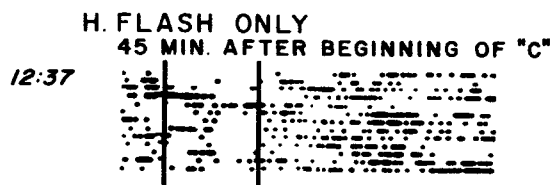
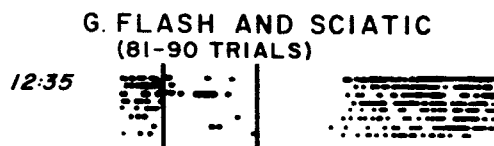
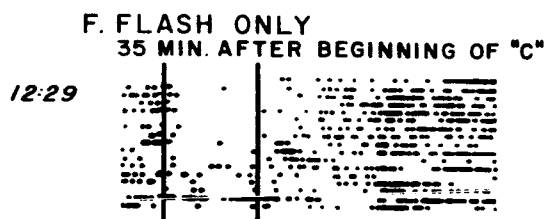
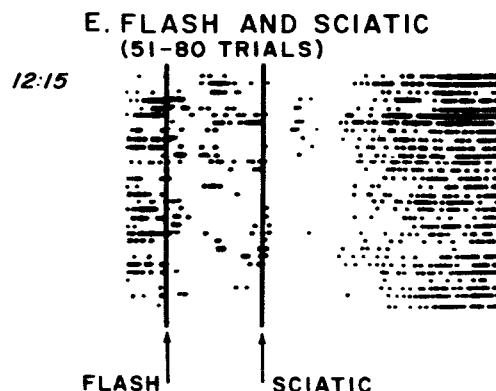
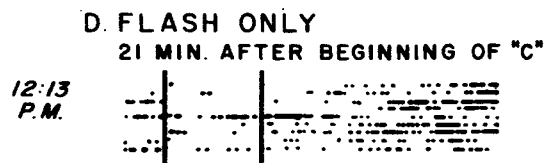
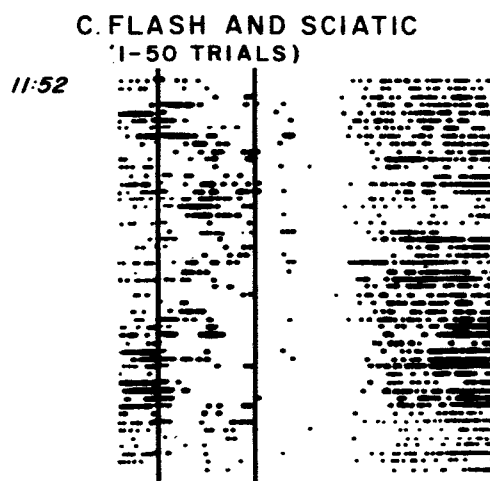


## LEVELS

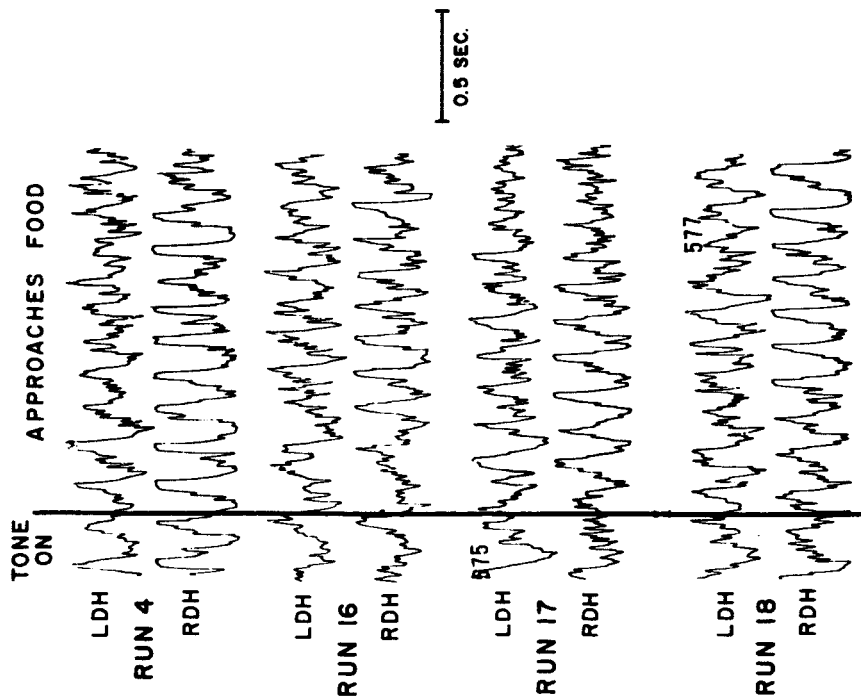
 $\Sigma = 0.40$  $\phi = 0.7$

# UNIT CONDITIONING - RIGHT N. HABENULAE LATERALIS

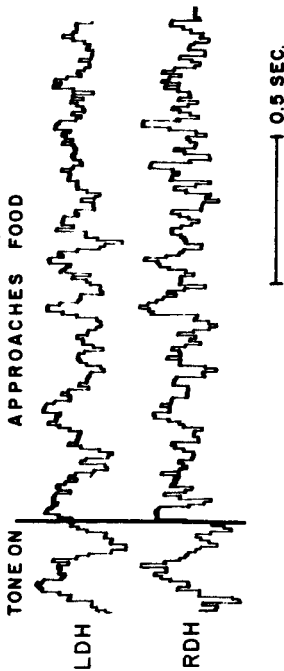
INTERTRIAL INTERVAL = 10 SEC.



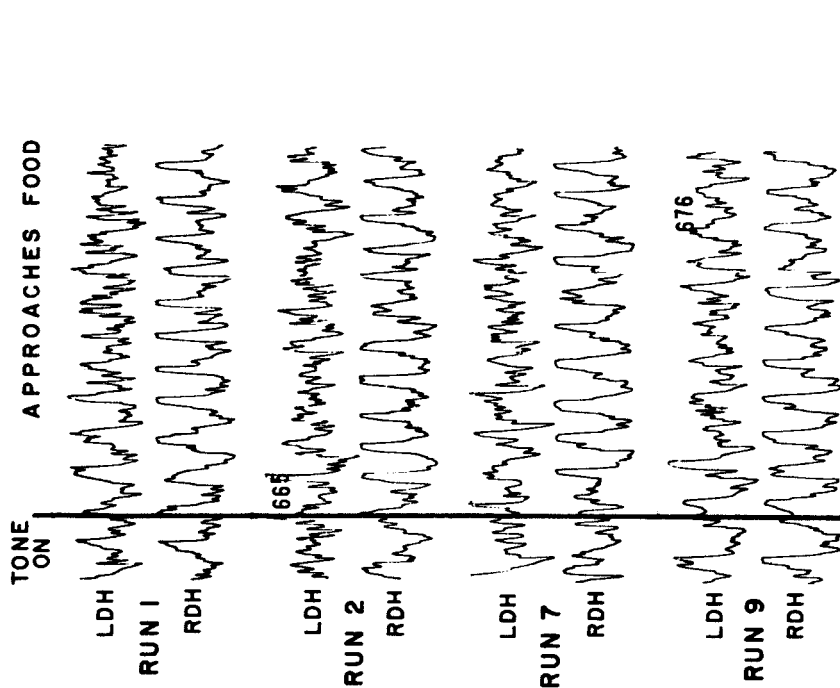
TRAINING 7 JULY 1962  
TRIAL 8: PERFORMANCE 75%



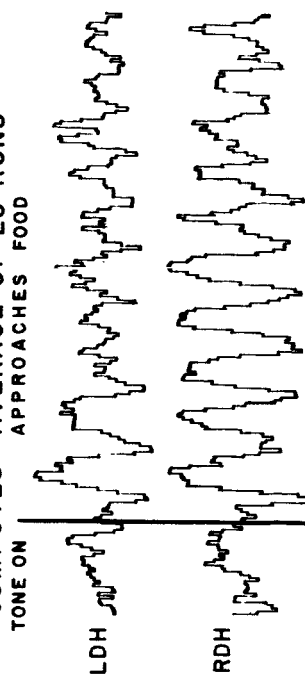
COMPUTED AVERAGE OF 20 RUNS



TRAINING 27 JULY 1962  
TRIAL 17: PERFORMANCE 78%



COMPUTED AVERAGE OF 20 RUNS

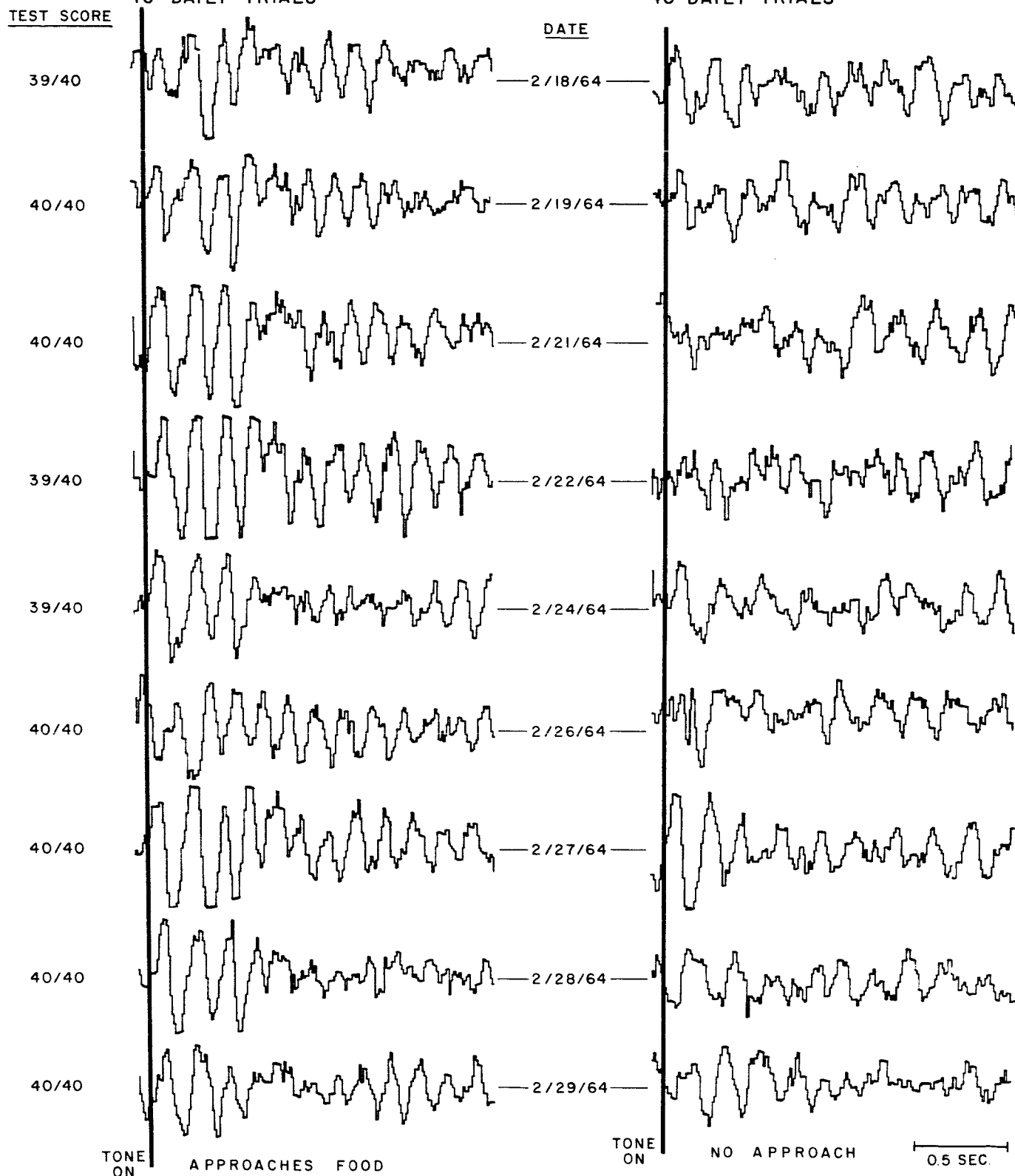


# COMPUTED HIPPOCAMPAL AVERAGES

CAT BP15

## A. AVERAGES DURING DISCRIMINATION 40 DAILY TRIALS

## B. AVERAGES DURING ORIENTATION WITHOUT DISCRIMINATION 40 DAILY TRIALS

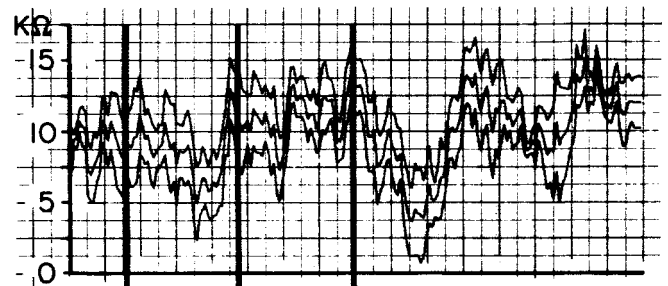
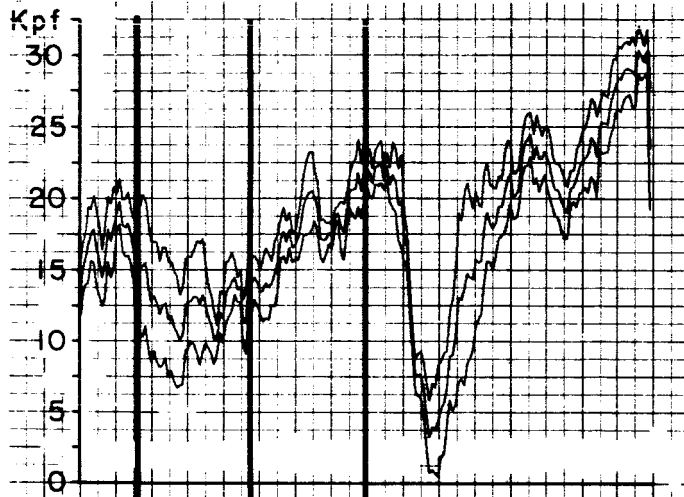


IMPEDANCE IN ALERTING, ORIENTATION AND DISCRIMINATION  
 COMPUTED MEANS WITH VARIANCE - AVERAGES FOR 5 DAYS  
 DORSAL HIPPOCAMPUS - CAT KAM 2

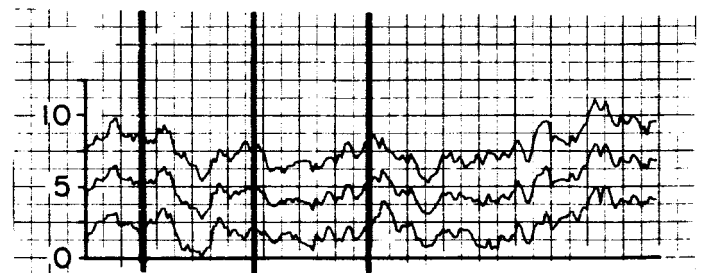
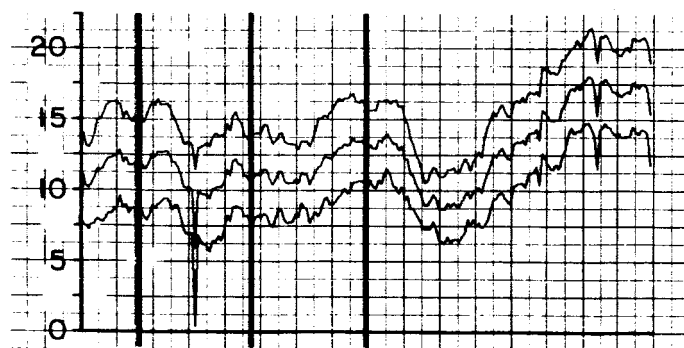
CAPACITANCE

RESISTANCE

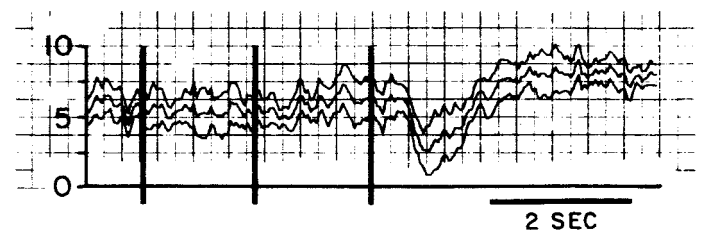
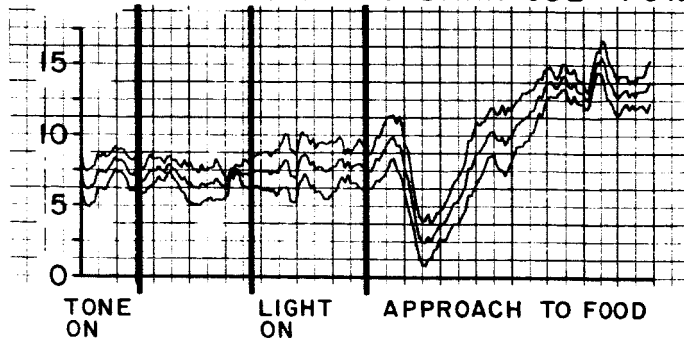
*A. 100% PERFORMANCE - LIGHT CUE*



*B. IMMEDIATELY AFTER CUE REVERSAL*



*C. RETRAINING TO DARK CUE - 76% PERFORMANCE*



# IMPEDANCE IN CAUDATE NUCLEUS EFFECTS OF $\text{Ca}^{++}$ IN RIGHT VENTRICLE

